FIG. 1

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Sheet 2 of 6
RENILLA REINFORMIS FLUORESCENT PROTEINS,
NUCLEIC ACIDS ENCODING THE FLUORESCENT
PROTEINS AND THE USE THEREOF IN
DIAGNOSTICS, HIGH THROUGHPUT SCREENING
AND NOVELTY ITEMS.
Applicant: Bryan et al.   Our Docket No.: 24729-0128
Serial No. 09/808,898   Filed: March 15, 2001

luciferase

480nm

FIG. 2A

luciferase

510nm

BRET

GFP

FIG. 2C

495nm

510nm

GFP

FIG. 2B

luciferase

480nm

ligand

GFP

FIG. 2D

BRET Sensor Architectures

15°   37°

optimized energy transfer module   simple conformational change

complex conformational change   association/dissociation

small molecule interference   large molecule interference

L luciferase

G GFP

protein domain

A antibody fragment

S small molecule

BRET sensors are depicted for
permissive and non-permissive binding
states of the target molecules.
Binding may be modulated by varying
temperature or ionic strength.

FIG. 3

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Utilization of advantageous GFP surfaces with
substituted fluorophores

```
                          60           *           80
RM-GFP   :   GAPLPFAFDIVSPAFQYGNRTFTKYPNDIS--   :   83
Pt-GFP   :   GGPLPFAFDIVSIAFQYGNRTFTKYPDDIA--   :   83
RR-GFP   :   GAPLPFAFDIVSVAFSYGNRAYTGYPEEIS--   :   80
cFP484   :   GAPLPFSYDILSNAFQYGNRALTKYPDDIA--   :   83
drFP583  :   GGPLPFAWDILSPQFQYGSKVYVKHPADIP--   :   80
asFP595  :   GGPLPFAFHILSTSCMYGSKTFIKYVSGIP--   :   77
dsFP483  :   GGPLPFGWHILCPQFQYGNKAFVHHPDNIH--   :   80
amFP486  :   GGPLAFSFDILSTVFKYGNRCFTAYPTSMP--   :   82
zFP506   :   GGPLPFAEDILSAAFNYGNRVFTEYPQDIV--   :   80
zFP538   :   GGPLPFSEDILSAGFKYGDRIFTEYPQDIV--   :   80
             ====================
```

FIG. 4

FIG. 5

R_reniform   ---MDLAKLGLKEVMPTKINLEGLVGDHAFSMEGVGEGNILEGTQEVKISVTKGAPLPFAFDIVSV   63
R_mullerei   MSKQI.KNTC.Q....SY.V-..I.NN..V.T.....C.K....F.N.L.Q.R........P   66
Ptilosarcu   MNRNV.KNT....I.SA.ASV..I.NN.V...F.K..V.F.N.LMQ.R....G......I   66
drFP583   ---.RSS.NVI..F.RF.VRM..T.NG.E.FI..E..RPY..HNT..LK....G.....W..L.P   63

AF[S]YGNRAYTGYPEE[I]SDYFLQSFPEGFTYERNIRYQDGTAIVKSDISLEDGKFIVNVDFKAKDL   129
.Q....TF.K..ND..I......A.M....TL..E...LVEIR..N.IED..VYR.EY.GSNF   132
.Q....TF.K..DD.A..V.....A.F...L.FE..AIVDIR...D..HYK.EYRQNGF   132
.Q.Q..SKV.VKH.AD.P..KKL....KW..VMNFE....VVT.TQ..S..Q...C..YK..K.IGVNF   129

RRMGPVMQQDIVGMQPSYESMYTNVTSVIGECIIAFKIQTGKHFTYHMRTVYKSKKPVETMPLYHF   195
PDD......KT.LIE..F..A..M.NGVLV..V.LVY..NS..YYSC..K.LM..GV.KEF.S...   198
PSN......KA.L..E..F..VV.M.SGVLV..VDLVY..ES.NYYSC..K.F.R..GG.KEF.E...   198
PSD......KKTM.WEA..T.RL.PRDGVLK..IHK.L..KD.G.YLVEFSKI.MA..APVQL..G.YY   195

00 IQHRI[V]K[T]NVDTASGYV[V]QHE[T]A[I]A[A]HSTIKK[I]E[G]SLP----[HEWV]   233
..   E..[Y.EDGGF-.E......QMTS.G.PL..E...[HEWV]   238
.H...E..[Y.EEG.F-..E......QLT..G.PL..E...[HEWV]   238
VDSK.[D]I.[S]HNEDYTI.E...Y.R[E]GR.HLFL----   226
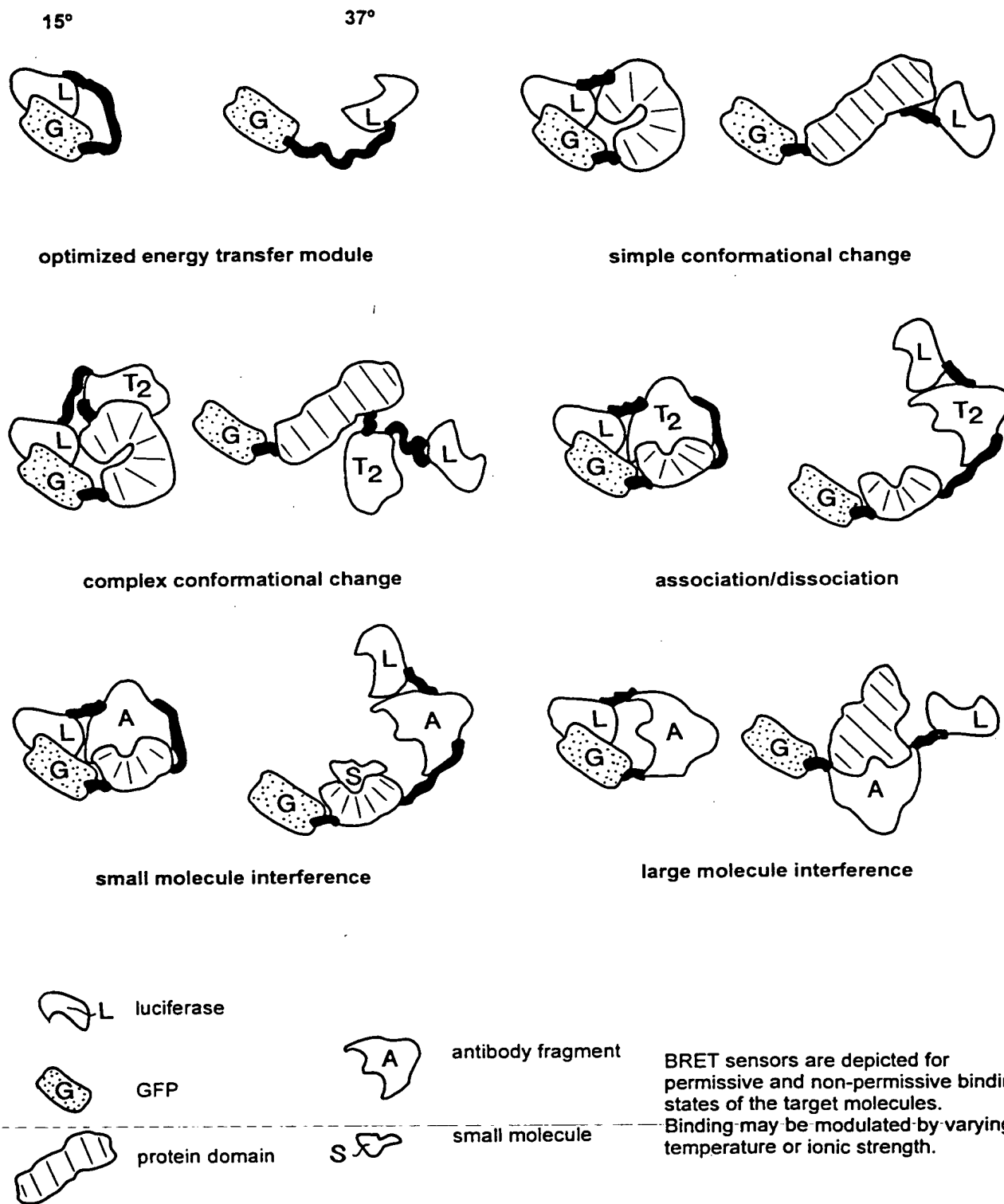
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FIG. 6

Block 1 (residues 1–80):

```
                           *         20        *         40        *         60        *         80
Aequorea   : --MSKGEELFTGVVPILVELDGDVNGHKFSVSGEGEGDATYGKLTLKFICTT---GKLPVPWPTLVTTFSYGVQCFSRYPDHMK :  79
R_mullerei : MSKQILKNTCLQEVMSYKVNLEGIVNNHVFTMEGCKGNILFGNQLVQIRVTK--GAPLPFAFDIVSPAFQYGNRTFTKYPNDI- :  82
Ptilosarcu : MNRNVLKNTGLKEIMSAKASVEGIVNNHVFSMEGFGKGNVLFGNQLMQIRVTK--GGPLPFAFDIVSIAFQYGNRTFTKYPDDI- :  82
R_reniform : --MDLAKLGLKEVMPTKINLEGIVGDHAFSMEGVGEGNILEGTQEVKISVTK--GAPLPFAFDIVSVAFSYGNRAYTGYPEEI- :  79
drFP583    : ---MRSSKNVIKEFMRFKVRMEGIVNGHEFFIEGEGEGRPYEGHNTVKLKVTK--GGPLPFAWDILSPQFQYGSKVYVKHPADI- :  79
drFP593    : ---MSCSKNVIKEFMRFKVRMEGIVNGHEFEIKGEGEGRPYEGHCSVKLMVTK--GGPLPFAFDILSPQFQYGSKVYVKHPADI- :  79
dsFP483    : ---MSCSKSVIKEEMLIDLHLEGIFNGHYFFIKGKGKGQPNEGTNTVTLEVTK--GGPLPFGWHILCPQFQYGNKAFVHHPDNI- :  79
cFP484     : ---KALTTMGVIKPDMKIKLKMEGNVNGHAFVIEGEGEGKPYDGCTHTLNLEVKMAEGAPLPFSYDILSNAFQYGNRALTKYPDDI- :  82
asFP595    : ---MASFLKKTMPFKTTIEGIVNGHYFKCTGKGEGNPFEGTQEMKIEVIE---GGPLPFAFHILSTSCMYGSKTFIKYVSGI- :  76
amFP486    : ---MALSNKFIGDDMKMTYHMDGCVNGHYFTVKGEGNGKPYEGTQTSTFKVTMANGGPLAFSFDILSTVFKYGNRCFTAYPTSM- :  81
zFP538     : ---MAHSKHGLKEEMTMKYHMEGCVNGHKFVITGEGIGYPFKGKQ--TINLCVIEGGPLPFSEDILSAGFKYGDRIFTEYPQDI- :  79
zFP506     : ---MAQSKHGLTKEMTMKYRMEGCVDGHKFVITGEGIGYPFKGKQ--AINLCVVEGGPLPFAEDILSAAFNYGNRVFTEYPQDI- :  79
```

Block 2 (residues 81–162):

```
                           *         100       *         120       *         140       *         160       *
Aequorea   : RHDFFKSAMPEGYVQERTIFFKDDGNYKTRAEVKFEG--DTLVNRIELKGIDFKEDGNILGHKLEYNYNSHNVYITADKQKNGIK : 162
R_mullerei : -SDYFIQSFPAGFMYERTLRYEDGGLVEIRSDINLIE--DKFVYRIVEYKGSNFPDDGPVMQKTI-LGIEPSFEAMYM--NNGVLV : 161
Ptilosarcu : -ADYFVQSFPAGFFYERNLRFEDGAIVDIRSDISLED--DKFHYKVEYRGNGFFSNGPVMQKAI-LGMEPSFEVVYM--NSGVLV : 161
R_reniform : -SDYFLQSFPEGFTYERNIRYQDGGTAIVKSDISLED--GKFIVNVDFKAKDLRRMGPVMQQDI-VGMQPSYESMYT--NVTSVI : 158
drFP583    : -PDYKKLSFPEGFKWERVMNFEDGGVVTVTQDSSLQD--GCFIYKVKFIGVNFPSDGPVMQKKT-RGWEASTERLYP--RDGVLK : 158
drFP593    : -PDYKKLSFPEGFKWERVMNFEDGGVVTVTQDSSLKD--GCFIYEVKFIGVNFPSDGPVMQKKT-RGWEASSERLYP--RDGVLK : 158
dsFP483    : -HDYLKLSFPEGYTWERSMHFEDGGLCCICIINDISLTG--NCFYDIKFYDIKFTGLNFPPNGPVVQKKT-TGWEPSTERLYP--RDGVLI : 161
cFP484     : -ADYFKQSFPEGYSWERTMTFEDKGICLVKVKSDISMEE--DSFIYEIHFQGVNFPPNGPVMQKKT-LKWEPSTEIMYV--RDGVLV : 155
asFP595    : -PDYFKQAFPDGMSYERTFTYEDGGVATASWEISLKG--DCLVYKVKIILGNNFPADGPVMAKKT-TGWDPSFEKMTV--VDGVLR : 160
amFP486    : -PDYFKNSCPAGYTWGRSFLEEDGAVCICNVDITVSVKENCIYHKSIFNGVNFPADGPVMKKMT-TNWEASCEKIMPVPKQGIIK : 162
zFP538     : -VDYFKNSCPAGYTWDRSFLFEDGAVCICNADITVSVEENCMYHESKFYGVNFPADGPVMKKMT-DNWEPSCEKIIPVPKQGIIK : 162
zFP506     :                                                                                        : 162
```

Block 3 (residues 163–238):

```
                           *         180       *         200       *         220       *         240
Aequorea   : VNFKIRHNIEDGSVQLADHYQQNTPIG-DGPVLLPDNHYLSTQSALSKDPNEKRDHMVLLEFVTAAGITHGMDELYK--- : 238
R_mullerei : GEVILVYKLNSGKYHSCHMKTIMKSKG--VVKEFPSYHFIQHRLEKTYVEDGGF--VEQHETAIAQMTSIGKPLGSLHEWV : 238
Ptilosarcu : GEVDIVYKLESGNYHSCHMKTFYRSKG--GVKEFPEYHFIHHRLEKTYVEECSF--VEQHETAIAQLITIGKPLGSLHEWV : 238
R_reniform : GECIIAFKLQTGKYFTYHMRTVYKSKK--PVETMPLYHFIQHRLVKTNVDTASG-YVVQHETAIAAHSTIKIEGSLP--- : 233
drFP583    : GEIHKALKLKDGGHYLVEFKSIYMAKK--APVQLPGYYYVDSKLDITSHNEDYT--IVEQYERTEGRHHLFL--- : 226
drFP593    : GDIHMALRLEGGGHYLVEFKSIYMVKK--PSVQLPGYYYVDSKLDMTSHNEDTY--VVEQYEKTQGRHHPFIKPLQ--- : 230
dsFP483    : GDIHHALTVEGGGHMACDIKTVYRAKK--AALKMPGYHFVDVIKLVIWNNDKEFM--KVEEHEIAVARHHPFYEPKKDK--- : 232
cFP484     : GDISHSLLLEGGGHYRCDFKSIYKAK--KVVKLPDYHFVDHRIEILNHDKDYN--KVTLYENAVARYSILPSQA--- : 231
asFP595    : GQSLMALKCPGGRHLTCHLHTTYRSKKPASALKMPGFHFEDHRIEIMEEVEKGK--CYKQYEAAVGRYCDAAPSKLGHN--- : 232
amFP486    : GDVTAFLMLQGGNYRCQFHTSYKITK---KPVTMPPNHVVEHRIARTDLDKGGN--SVQLTEHAVAHITSVVPF--- : 229
zFP538     : GDVSMYLLLKDGGRYRCQFDTVYKAK--SVPSKMPEWHFIQHKLLREDRSDAKNQKWQLTEHAIAFPSALA--- : 231
zFP506     : GDVSMYLLLKDGGRYRCQFDTVYKAK--SVPRKMPDWHFIQHKLTREDRSDAKNQKWHLTEHAIASGSALP--- : 231
```

Legend:

dimerization surfaces   □ hydrophilic   ☐ hydrophobic

| D,E,H,K,R polar charged | N,Q,S,T polar uncharged | L,I,V,M,F,Y,W non-polar hydrophobic | A,G small | C,P not grouped |